

Original papers

Toxoplasma gondii and women of reproductive age: an analysis of data from the Chair of Microbiology, Jagiellonian University Medical College in Cracow[1]

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ABSTRACT. The aim of the study is to determine the seroprevalence and risk factors for *Toxoplasma gondii* infection in women of reproductive age within the Małopolska province who were examined in the Laboratory of Microbiological Diagnostics, Chair of Microbiology in Cracow in 2013–2014. Seventy-eight questionnaires completed by women of reproductive age (mean age 29.91±6.56) were analysed. In total, 82% of respondents signed up for serological testing while already pregnant (33 patients in the age group 18–29 years and 30 patients in the age group 30–39 years). Twenty-seven percent had no previous contact with the parasite. A statistically significant (chi-square=7.722, p=0.005) correlation between permanent residence in the countryside and the presence of anti-*T. gondii* antibodies was found. A significant correlation was shown in the studied group between a lack of contact with soil and negative results of serological tests for toxoplasmosis (chi-square=4.116, p=0.042). The majority of the surveyed women had already encountered this parasite. It seems that special attention should be given to rural women. Implementation of more precise testing in the diagnosis of toxoplasmosis is also essential.

Key words: *T. gondii*, women, reproductive age, Poland

Introduction

Toxoplasmosis is considered one of the most widespread zoonoses in the world. It is caused by the protozoan *Toxoplasma gondii*. While members of the family Felidae are the definitive hosts, certain bird species and mammals, including humans, serve as intermediate hosts. Humans can become infected by any of the following: 1. digestion (ingesting parasites on the hands, fruit, vegetables, water, soil or sand contaminated with *Toxoplasma gondii* oocysts, as well as raw or half-raw meat containing cysts); 2. transplacentally (passed from mothers with acute infection in pregnancy onto their unborn children); 3. blood transfusion and transplantation of tissues containing tachyzoites [1,2].

Most frequently, toxoplasmosis is asymptomatic in infected hosts. The basis for diagnosis is a detailed case history detailing exposure to factors favoring infection, as well as serological testing. The detection of different classes of antibodies (IgM, IgG, IgA and IgE) resulting from invasions and the estimation of their titer are commonly used to determine whether the host has come into contact with the parasite. The antibody assay indicates the presence of a newly-acquired or an old infection, which can be distinguished by an IgG antibody avidity assay. Determination of these facts is particularly important in patients with a dysfunction of the immune system. Toxoplasmosis in such cases may take the form of severe neurological symptoms. [1,2]

Regular updates and verification of the epidemiological situation regarding toxoplasmosis is vital in the context of *Toxoplasma gondii* infection, not only in immunocompromised patients, but also in women of reproductive age, because the primary, active form of toxoplasmosis

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in women during pregnancy poses a danger of infection to the unborn child and the development of congenital parasitosis, from miscarriage, through serious defects of the central nervous system in the newborn, to various degrees of chorioretinitis later in life. [3,4].

Attempts to determine the exact number of people infected and routes of transmission of the parasite are being made. The results of serological testing indicate that infection with *Toxoplasma gondii* occurs in almost all species of warm-blooded animals and in approximately one-third of the world population. The highest rates of infection are recorded in inhabitants of temperate and warm climate zones, and in countries where there is a custom of eating dishes prepared from raw or undercooked meat [1,2].

The aim of the present study is to determine the seroprevalence and risk factors for infection with the protozoan *Toxoplasma gondii* based on the presence of specific antibodies among women of reproductive age of the Małopolska province who reported for examination in 2013–2014.

Materials and Methods

The serological test results for the presence of anti-*T.gondii* antibodies in the subjects was obtained from the Laboratory of Microbiological Diagnostics of the Chair of Microbiology, Jagiellonian University Medical College (JUMC) in Cracow for the period 1st January 2013 to 15th March 2014. A specially-devised questionnaire pertaining to the risk factors of *T.gondii* infection was administered to the subjects, all of whom reported to the Chair of Microbiology for serological testing for toxoplasmosis and agreed to participate in the study (consent for research of the Bioethics Committee of the Jagiellonian University no. KBET/272/B/2012).

The following responses to the survey questions were included in the analysis: age, gynecological parameters (pregnancies, miscarriages), place of residence, eating habits and hygiene habits (eating raw meat, washing raw vegetables and fruits before eating, washing hands during food preparation, contact with soil), and ownership of animals. For each group of parameters, a preliminary statistical analysis was performed. For further processing of the obtained data, a chi-square test was employed. Differences at a confidence level of $p<0.05$ were considered statistically significant.

Results

In the period from 1st January 2013 to 15th March 2014, 664 people entered the study and underwent serologic testing for toxoplasmosis at the JUMC Laboratory of Microbiological Diagnostics, Chair of Microbiology. This number comprised 209 children (31.5%) and 455 adults, including 11 men (1.5%) and 444 women (67%). Among the women, 439 (99% of all women, 66% of all patients studied), were aged between 18 to 49 years, i.e. of reproductive age. In 67% of these respondents, the presence of either IgM or IgG anti-*T.gondii* antibodies was found.

In total, 78 questionnaires were completed by the female respondents of reproductive age (mean age 29.91 ±6.56). Forty were in the 18–29 age group, 36 in the 30–39 age group, and 2 in the 40–49 age group. The presence of IgM antibodies for toxoplasmosis was detected in 3% of these women (2 people, both of whom were between the ages of 18 and 29). Both IgM and IgG together were identified in 15% (12 patients), and IgG alone in 50% (37 women). No antibodies, and hence, lack of contact with *Toxoplasma gondii*, was recorded in 32% of the studied patients (25 people).

The majority (82%) of women from the analyzed group signed up for serological testing while already pregnant: 33 patients in the 18–29 age group and 30 in the 30–39 age group. Seventeen of them (almost 27%) reported no prior contact with the parasite and needed to be monitored until the completion of their pregnancies. In one patient, only IgM antibodies were detected, which indicated the acute phase of toxoplasmosis. Eight people (12.6%) demonstrated both IgM and IgG antibodies, which did not allow the phase (active or chronic) of the infection to be clearly determined. IgG antibodies were found in 37 women (58.7%) which indicated a chronic form of toxoplasmosis. Additional examination of the binding strength of IgG antibodies (avidity) in the 2 latter groups established that 29 people were in the chronic phase of parasitosis (high avidity ≥60%). However, it was not possible to determine the phase of infection in eight women with IgG antibodies and in one patient with IgM and IgG antibodies, as the avidity value was in the gray zone, while acute toxoplasmosis was suspected in the case of one person with IgM and IgG, as avidity was lower than 30%).

Further analysis of the 78 questionnaires showed that the most common place of residence, was the

city, with 36 people (46%) stating they lived there. However, 26 women (33%) declared living in the countryside, of whom 24 claimed that they had not moved during their lifetime. A statistically significant (chi-square=7.722, p=0.005) correlation was also demonstrated between permanent residence in the countryside and the presence of anti-*T.gondii* antibodies (Fig. 1).

Questions regarding contact of the skin on the hand with soil, when working on the land, in the garden, or while taking care of potted plants, generated 38 affirmative answers (49%) and 40 negative answers (51%). Statistical analysis of the data (chi-square=4.116, p=0.042) revealed a significant correlation between lack of contact with soil and negative results of serological tests for toxoplasmosis (Fig. 2).

In addition, 44 patients (56%) reported eating meals prepared from raw meat, 34 women (43.5%) claimed that they sometimes ate unwashed fruits and vegetables, and only 7 patients (9%) drank untreated water. On the other hand, 71 people (91%) reported always washing their hands before food preparation. No statistically significant correlations were found between the results of serological testing for anti-*T.gondii* antibodies and the above-mentioned variables.

A similar lack correlation was found between serological status and possession of animals. Neither possession of a dog (14 respondents had always had one, 17 only currently, 26 only in childhood) nor a cat (40 people reported never owning one) had any statistically significant relationship with serological status.

Discussion

The presented analysis demonstrates that at the JUMC Laboratory of Microbiological Diagnostics of the Chair of Microbiology in Cracow in 2013–2014, serological testing for toxoplasmosis was conducted most frequently in women of reproductive age, followed by children. Verification of infection with the parasite *Toxoplasma gondii* these specific groups of patients stems from the risk that acute toxoplasmosis poses to pregnant women [3,4].

The presence of anti-*T.gondii* antibodies was found in as many as two-thirds of examined women of reproductive age, which indicates that they had previously come into contact with this protozoan. In Poland, based on data concerning infection with *Toxoplasma gondii* from studies in different years and in selected regions of Poland, most often concerning groups at risk, such as pregnant women or persons having professional contact with soil or animals, the percentage of seropositive adults can be estimated at 40–50% [5–10]. Such a high percentage of seropositive patients in this study can be explained by the fact that the women referred to our Laboratory, as a reference center, had been subjected to previous examinations but required verification as regards the phase, either active or chronic, of the infection.

The Sabin–Feldman dye test (DT) can be used to clarify such ambiguous results. This test is still considered to be the gold standard for the diagnosis of toxoplasmosis due to its high sensitivity and specificity, although it does not differentiate classes of antibodies. As it is a time-consuming examination requiring living cells of the parasite supplied with a complement from an appropriately immunized donor, it is very rarely used nowadays. A different method is thus widely used in pregnant women, the avidity test. This is a study evaluating the binding strength of IgG antibodies: the longer the duration of infection, the greater the binding strength of antibodies to antigens of the parasite. [11,12]. However, the findings of the present study indicate that it is not always possible to clearly determine the phase of infection with the use of this method: nine pregnant women presented avidity values within the so-called gray zone.

Pregnant women in whom serologic tests for toxoplasmosis turned out to be negative are particularly noteworthy. Despite typically not ever having contact with *Toxoplasma gondii*, they are vulnerable to developing the active form of parasitosis when infected with the protozoan. If this occurs during pregnancy, it can lead to the development of congenital toxoplasmosis in the child. The risk of vertical transmission increases with gestational age: it is greatest in the third trimester, with the likelihood ranging from 60 to 81% [3,4,10]. Women with no anti-*T. gondii* antibodies, therefore, require regular serological testing during pregnancy. In some countries (France), they are mandatory every month, which allows seroconversion to be identified quickly and for appropriate measures to be taken, such as making use of specific treatment [13]. In Poland, such tests are only recommended by the Minister of Health Regulation of 4 October 2012 on standards of conduct and medical procedures in providing

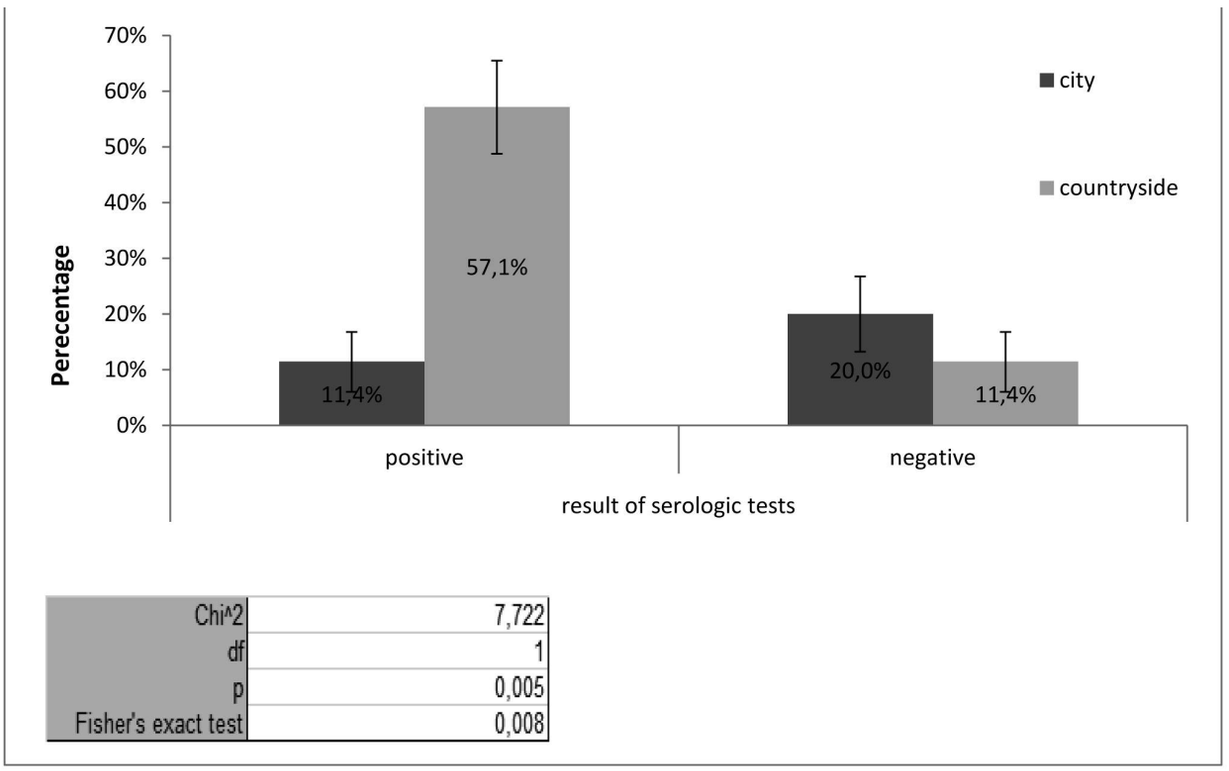

Fig. 1. Statistically significant correlation between permanent (no moves in lifetime) residence in the countryside and the presence of anti-*T. gondii* antibodies

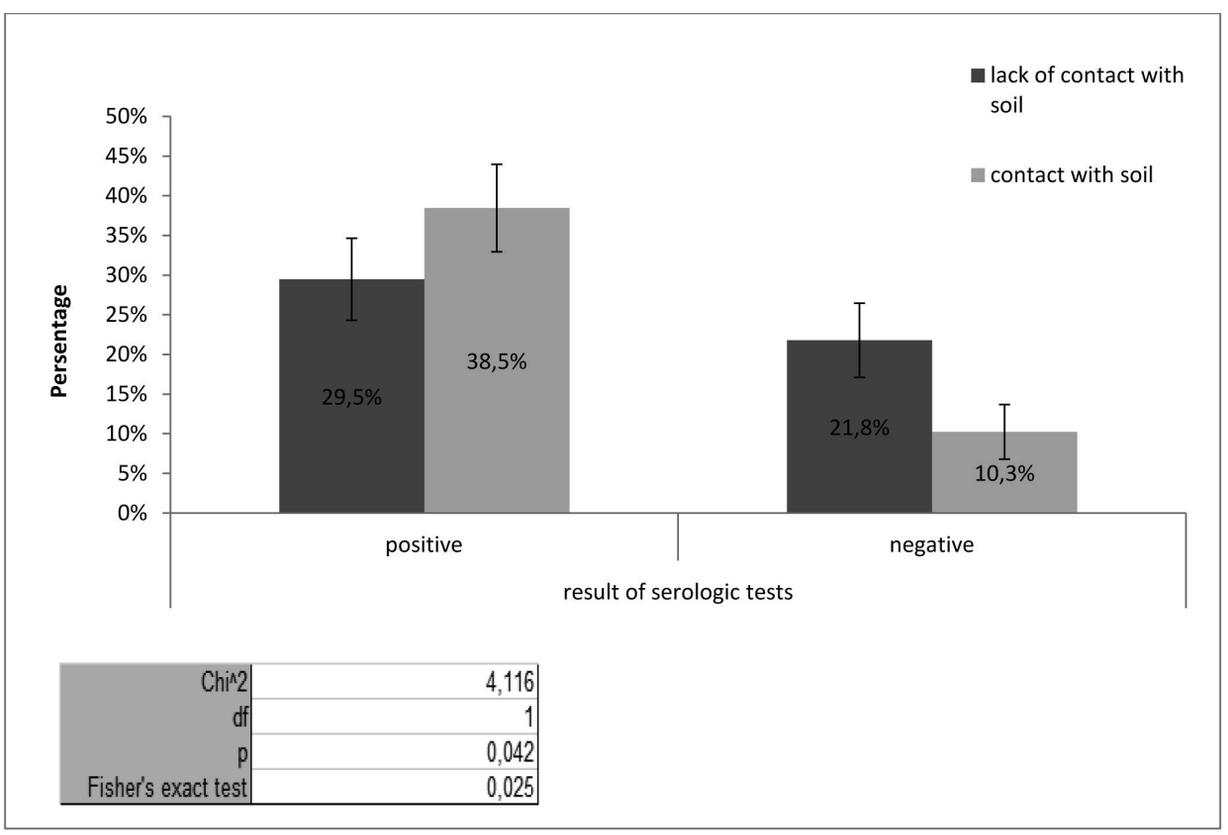

Fig. 2. Statistically significant correlation between lack of contact with soil and negative results of serological tests for toxoplasmosis

healthcare services in the field of perinatal care exercised over women during physiological pregnancy, physiological childbirth, puerperium and infant care [14], but only in two situations: until the 10th week of pregnancy and if they are negative, at 21–26 weeks pregnant. Additionally, there is no nationwide program of congenital toxoplasmosis prophylaxis. Hence, education of seronegative women during pregnancy plays an enormous role. Knowledge of routes of transmission and risk factors allows the presentation of behaviors that will protect against infection with *Toxoplasma gondii.*

The findings hereing indicate that women living in rural areas require special care in this respect, especially those who are in contact with soil, which may be contaminated with oocysts of the parasite. Although our observations do not conclusively indicate the influence of diet and hygiene behaviors as being responsible for infection, many publications stress the fact that the consumption of dishes prepared from meat which is raw or not subjected to appropriate heat treatment, such as raw sausage or shellfish, which are gaining popularity in Poland, or unwashed raw vegetables and fruits, is a major risk factor for toxoplasmosis. [1,2,4,10,15].

Both the results of the study discussed above and the observations of other authors indicate that the mere possession of animals is not explicitly associated with an increased risk of contracting this protozoan. Felines, especially domestic cats, are the well-known definitive hosts of *Toxoplasma gondii*. Although the cats deposit fecal oocysts in the soil, sand, grass and water, it is believed that it is unlikely that the cats which stay at home, do not hunt, or eat raw meat, could have been infected. Dogs can act only as potential mechanical vectors: the dog's fur and paws can be contaminated with oocysts [2,4,15].

Knowledge of the biology of *Toxoplasma gondii*, its routes of infection and diagnostic methods are of particular importance in the care of pregnant women. Unfortunately, in Poland, there is no mandatory mass screening for neither the general population nor pregnant women for the presence of antibodies against *Toxoplasma gondii*. The obligation for doctors to notify the health inspectorate of cases of toxoplasmosis was limited solely to symptomatic cases of congenital toxoplasmosis [10,16]. Therefore, the data available concerning the parasitosis is incomplete and restricted to selected regions.

Conclusions

The consequences of infection with *Toxoplasma gondii* in the perinatal period were the most common cause of testing for toxoplasmosis in 2013–2014 at the Laboratory of Microbiological Diagnostics of the Chair of Microbiology, JUMC in Cracow.

Most of the women studied in this period had already come into contact with *Toxoplasma gondii*.

Special attention (research, monitoring, education) should be paid to women living in rural areas.

More precise tests such as the use of *T. gondii* recombinant antigens and their use as molecular markers of toxoplasmosis should be provided in the diagnosis of toxoplasmosis to determine the phase of infection with the protozoan during pregnancy and assess the risk of congenital toxoplasmosis in the child.

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